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13. ABSTRACT ***(Maximum 200 Words)***

Nuclear receptors undergo conformational changes when they bind their cognate ligands. It should be possible to monitor these changes *in vivo* using resonance energy transfer between fluorophores. The existence of inherently fluorescent proteins such as the variants of jellyfish green fluorescent protein (GFP) suggests that this problem may be approached by making fusions of these proteins to nuclear receptors. We set out to study this problem using the estrogen receptor (ER), a nuclear receptor known to undergo a conformational change upon ligand binding. We have proposed to generate a novel intrinsic ligand binding assay for the estrogen receptor based on ligand dependent conformational changes detected by fluorescence resonance energy transfer (FRET) between complimentary fluorescent proteins. We are in the process of cloning double and single chimeras of the estrogen receptor and the various fluorescent proteins into mammalian CMV expression vectors. We have extended the number of chimeras that we are generating because of the advent of new fluorescent proteins now available from Clontech, which include cyan, yellow and red fluorescent protein vectors. These new fluorescent proteins are more optimal for FRET than the original blue and green variants.

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FOREWORD

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Arthur Chung 7/11/03

PI - Signature Date

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Introduction

Nuclear receptors undergo conformational changes when they bind ligands. It should be possible to monitor these changes *in vivo* using energy transfer between fluorophores. The existence of inherently fluorescent proteins such as the variants of jellyfish green fluorescent protein (GFP) suggests that this problem may be approached by making fusions of these proteins to nuclear receptors. We set out to study this problem using the estrogen receptor (ER), a nuclear receptor known to undergo a conformational change upon ligand binding. The proposed assay we have set out to develop is shown in Fig. 1.

Summary of Progress

Training:

I have gained much needed training in many areas of molecular biology including subcloning, protein expression, transfection of mammalian cell lines, and reporter assays. In addition, I am gaining biochemical training using hormone binding assays.

Accomplishments:

Technical Objective 1:

Task 1:

Initially I proposed to create estrogen receptor (ER) chimeras with blue fluorescent protein (BFP) and green fluorescent protein (GFP) to generate a novel ligand binding assay based on fluorescence resonance energy transfer (FRET) between the two fluorescent reporters. I am in the process of subcloning the GFP ER BFP chimeras into a CMV expression vector for our cell based studies. However, since this proposal was submitted several other fluorescent proteins became available that have greater efficacy for FRET than GFP and BFP. Other jellyfish fluorescent protein vectors became available from Clontech that are mutants of the original GFP and thus have different spectral properties. They include the yellow and cyan fluorescent proteins (YFP and CFP, respectively), which have better complimentary excitation and emission peaks than do BFP and GFP, allowing clearer distinction of FRET (Fig. 2A and B). In addition, this past year Clontech has commercialized some new coral fluorescent proteins, one of which emits in the red part of the spectra and is thus called red fluorescent. RFP will form a good FRET partner with either GFP and CFP, as their emission peaks overlap with the excitation peak of RFP (Fig. 2A and B)

Thus, I wanted to take advantage of these new fluorescent reagents, which will increase the likely of successfully establishing ligand dependent FRET. I have commenced generating a new set of ER chimeras. Double chimeras with a FP at the N-terminus and the complimentary FP at the C-terminus will be created for the cis

assay and single chimeras, which will allow mixing and matching, are being made to establish the assay in trans as well. The following chimeras are being generated:

G:ER:R	C:ER:R	C:ER:Y	
R:ER:G	R:ER:C	Y:ER:C	
G:ER	C:ER	R:ER	Y:ER
ER:G	ER:C	ER:R	ER:Y

Task 2:

To be initiated.

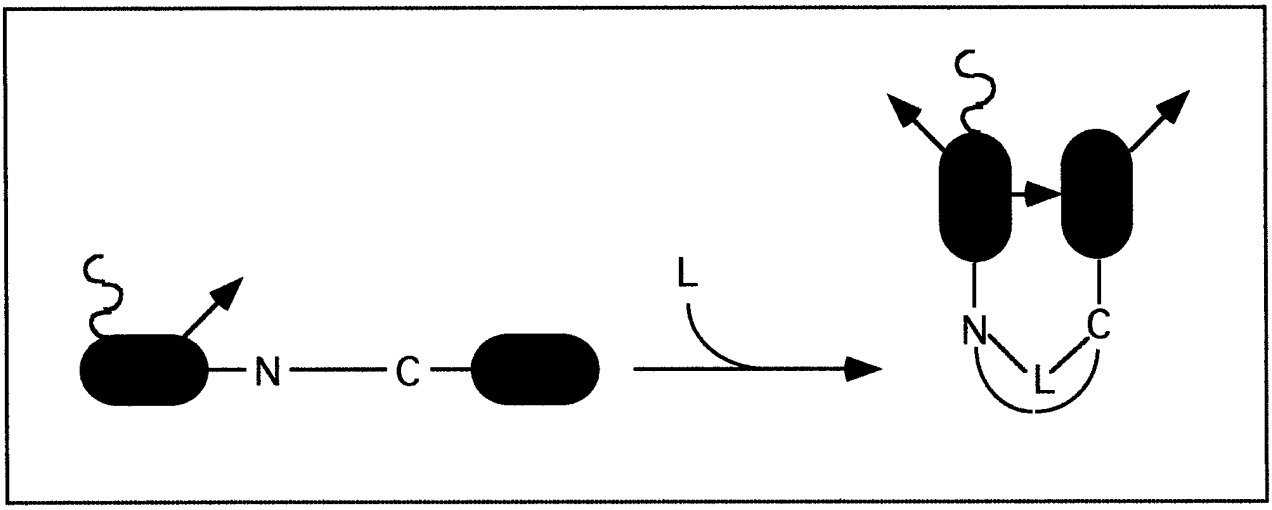

Figure 1: Ligand dependent steroid receptor assay based on FRET detection of conformational changes in the receptor upon hormone binding.

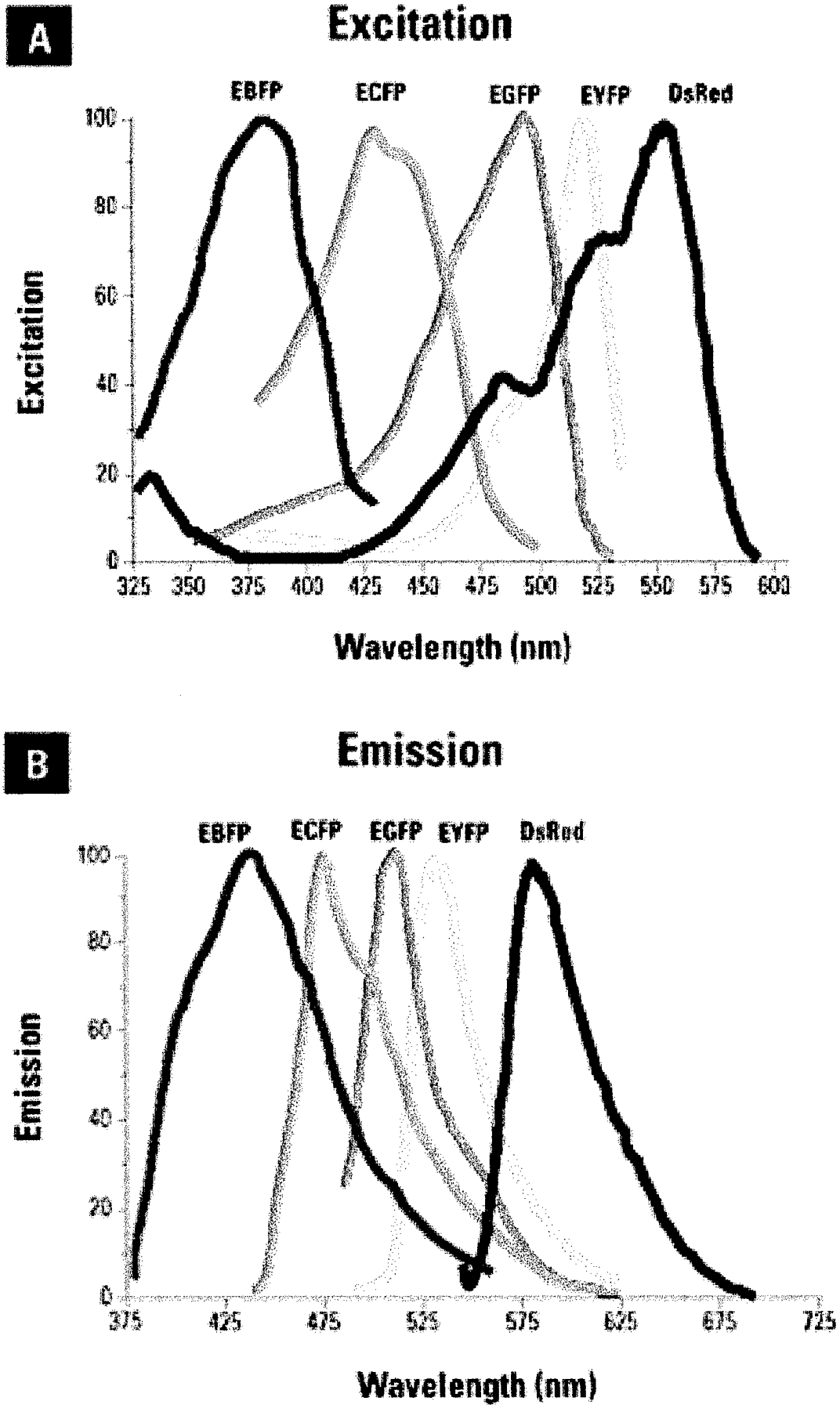

Figure 2: Excitation (A) and emission (B) spectra for the fluorescent proteins

Appendix

Key Research Accomplishments:

- Subcloning of ER fluorescent protein single and double chimeras.

Reportable Outcomes:

- **Research disclosure applied for.**